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Geographic origins and phylogenetic affinities of the putative Hawaiian endemic *Rhodocollybia laulaha*

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Abstract — The Hawaiian mushroom *Rhodocollybia laulaha* was selected as a model to investigate patterns of gene flow between geographically isolated fungal populations from ecologically and bioclimatically varied sites. Its morphology (distinctive when compared to other members of the genus) and affinity for endemic Hawaiian forest suggested that it was endemic to Hawaii. However, speculation as to its closest non-Hawaiian relative and its overall placement within the genus was based on mostly anecdotal evidence. The present morphological and genetic research identifies a well-supported clade comprising *R. laulaha* individuals from across the Hawaiian Islands, reveals *R. lignitilis* (described in 2004 from the Neotropics) to be conspecific with *R. laulaha,* and identifies *R. unakensis* from Texas as a putative sister taxon. Different possible historical scenarios are discussed regarding the migration and establishment of *R laulaha* ancestors between the Americas and Hawaii. *Rhodocollybia lignitilis* is synonymized with *R. laulaha,* and *Marasmius clavipes* is transferred to *Rhodocollybia*.

Key words — ITS, LSU, species range

Introduction

Rhodocollybia laulaha Desjardin et al. was described from the Hawaiian Islands in 1999. At that time, it was recognized as morphologically distinct

from other known *Rhodocollybia* species in having pale-orange to grayish-orange, labyrinthine, constricted lamellae (Desjardin et al. 1999). Its specific epithet 'laulaha' is the Hawaiian word for 'common and widespread'. Indeed *R. laulaha*'s range extends from the northwesternmost coast of Kauai to the southeasternmost corner of the Big Island and is present on all major islands in between. It fruits prolifically from July through December with peak mushroom production in August and September. In an analysis of the phenology and abundance of several putative Hawaiian endemic mushrooms, *R. laulaha* proved the most prolific mushroom producer of the taxa studied (Hemmes & Desjardin 2002). While its range extends the entire length of the modern Hawaiian Islands, it is significantly restricted by habitat. The forest habitat to which *Rhodocollybia laulaha* is limited (montane wet forest, montane mesic forest, lowland mesic forest, and lowland alien forest) is highly fragmented on the Hawaiian Islands creating a spatially subdivided system with forest 'islands' distributed across oceanic islands.

Rhodocollybia is a small genus with 35 species and subspecies described for the genus in online fungal databases (Farr et al. 2005) and an additional six neotropical species recently described from Costa Rica and Panama (Mata et al. 2004). Phylogenetic analyses utilizing nuclear large subunit (nLSU) and internal transcribed spacer (ITS) gene regions suggest that the genus *Rhodocollybia* is monophyletic and is most closely related to members of the genus *Lentinula* (Wilson & Desjardin 2005). Members of the genus *Rhodocollybia* are broadly distributed throughout temperate regions of North America and Europe and montane regions of Central America. A single *Rhodocollybia* species was described from Indonesia (*Rhodocollybia spissa* (A.W. Wilson et al.) A.W. Wilson & Desjardin; Wilson & Desjardin 2005), a single species from Thailand (*Marasmius clavipes* ⊠ *Rhodocollybia clavipes*), and a single *Rhodocollybia* of uncertain specific identity has been reported from South Africa (van der Westhuizen & Eicker 1994, as *Collybia distorta* (Fr.) Quél.). A taxon similar in appearance to *Rhodocollybia butyracea* (Bull.) Lennox is common in Australia (G.M. Mueller, pers. com.). A phylogenetic reconstruction using nLSU data placed *Rhodocollybia laulaha* in the monophyletic clade containing other *Rhodocollybia* species from the New World (Wilson & Desjardin 2005).

Support for the populations of *R. laulaha* belonging to a single species endemic to the Hawaiian Islands was based solely on its morphological distinctiveness and its reliable association with endemic Hawaiian rain forest vegetation (Desjardin et al. 1999). Understanding of the role of long distance spore dispersal in the maintenance of fungal species cohesion is in its infancy. Some evidence suggests that fungal spores are seldom dispersed for distances greater than 100 meters indicating that despite rare long distance dispersal events, significant gene flow via spore dispersal even between islands within

Hawaii is quite unlikely (Bergemann & Miller 2002, Burnett 2003). Other evidence suggests that a single fungal species can sustain appreciable gene flow across virtually global distributions (James et al. 2001, Petersen & Hughes 2007), but the dispersal mechanisms in such cases remain unclear.

The possibility exists that a putatively endemic Hawaiian taxon like *R. laulaha* does not actually represent a single lineage but rather the descendents of multiple independent introductions. Global phylogeography studies of the upside-down jellyfish genus *Cassiopea* using mitochondrial haplotype data suggest that two species of *Cassiopea* within the Hawaiian Islands represent independent introductions during the last 100 years – one from the Indo-Pacific, the other from the Red Sea/Atlantic. Genetic data indicate that the two species of *Cassiopea* currently occupying the island of Oahu are separated by 14–40 million years of reproductive isolation despite nearly identical morphology (Holland et al. 2004).

The goal of the present study was to determine whether or not *R. laulaha* represents several lineages with independent introductions to the Hawaiian Islands or a single lineage and single migration event to Hawaii. Additionally, we sought to identify a potential geographic source for the ancestor(s) of *R. laulaha* and to estimate the number of introductions if more than one. This type of search for a 'sister taxon' is difficult, especially for organisms such as fungi with largely unknown distributions. A recent estimation of worldwide macrofungal diversity calculated only 16–41% of macrofungi to be known to science and that endemism levels for macrofungi may be as high as 40–72% (Mueller et al. 2007). Considering that there is an extreme paucity of data regarding native species of macrofungi from most global regions outside of Europe and North America, it is safe to say that our knowledge of fungal diversity and distribution is minimal.

Investigations of other taxonomic groups have led to hypotheses on the progenitors of Hawaiian radiations: members of the plant bug genus *Sarona* in Hawaii represent radiation of a single introduction from the Americas (Asquith 1995); the spectacular honeycreeper radiation appears to be the sister group to a New World cardueline finch (*Carpodacus mexicanus*) whose common ancestor traveled to Hawaii roughly 3.5 million years ago (Tarr & Fleischer 1995); and the well-known Hawaiian silversword alliance members are descendants of a single California tarweed migrant that moved to Hawaii probably about 5 million years ago (Baldwin & Robichaux 1995). Nevertheless, the sister clade of many Hawaiian radiations remains unclear including that of the large Hawaiian *Drosophilidae* radiation, whose common ancestor may have arrived in Hawaii before formation of the oldest modern high island of Kauai (Desalle 1995). Members of the spider genus *Tetragnatha* in Hawaii are thought to represent at least two independent origins in Hawaii (Gillespie et al. 1994). [Note: Some

Table 1. *Rhodocollybia* species and outgroup taxon included in the analysis of ITS sequence data.

Species	Herbarium*	Collection ID	Geographic Origin	GenBank Accession
G. dryophilus (outgroup)	TENN	57012	Macon, Co., NC	DQ241781
R. amica	TENN	56662	Costa Rica	AF505754
R. butyracea	TENN	55660	Turkey	AY313289
R. butyracea	TENN	56303	Mexico	AY313290
R. butyracea	TENN	59317	Austria	AY313291
R. butyracea		PL 33	Czech Republic	EF062462
R. butyracea	TENN	55660	Turkey	AY256689
R. butyracea	TENN	53580	Sweden	AY313293
R. butyracea		cult. 8250	USA	AY313292
R. butyracea		OKM 2756	USA	DQ444317
R. clavipes	SFSU	DED 8151	Thailand	GU369941
R. dotae	NY	REH 7007	Costa Rica	AF505758
R. laulaha	SFSU	DEH 61492	Maui, HI	GU369942
R. laulaha	F	MRK 56	Big Island, HI	GU369943
R. laulaha	SFSU	DED 6393	Kauai, HI	GU369944
R. laulaha	F	MRK 57	Big Island, HI	GU369945
R. laulaha	F	MRK 58	Maui, HI	GU369946
R. laulaha	SFSU	DEH 502	Big Island, HI	GU369947
R. laulaha	SFSU	DEH 482	Big Island, HI	GU369948
R. laulaha	SFSU	DEH 847	Big Island, HI	GU369949
R. laulaha	SFSU	DEH 600	Kauai, HI	GU369950
R. laulaha	F	MRK 50	Big Island, HI	GU369951
R. laulaha	F	MRK 52	Big Island, HI	GU369952
R. laulaha	SFSU	DEH 952	Kauai, HI	GU369953
R. laulaha	SFSU	DEH 004	Kauai, HI	GU369954
R. laulaha	F	MRK 53	Big Island, HI	GU369955
R. laulaha	F	MRK 51	Big Island, HI	GU369956
R. laulaha	F	MRK 54	Big Island, HI	GU369957
R. laulaha	F	MRK 55	Big Island, HI	GU369958
R. lignitilis	NY	REH 7907	Panama	AF505753
R. lignitilis	TENN	56628	Costa Rica	GU369959
R. maculata	TENN	59459	USA	AY256688
R. maculata	TENN	59459	USA	AY313296
R. maculata	CFH	AFTOL ID 540	USA	DQ404383
R. maculata	TENN	56568	USA	AY313297
R. pandipes	TENN	59546	Dominican Republic	AY313288
R. pandipes	TENN	53838	Costa Rica	AY313294
R. prolixa	NY	EFM 1403	Costa Rica	AF505748
R. tablensis		EN 2066	Costa Rica	AF505755
R. turpis	TENN	58017	Costa Rica	AF505749
R. unakensis	TENN	58545	Beaumont, TX	AY313298

* TENN = University of Tennessee; SFSU = Harry D. Thiers Herbarium, San Francisco State University; NY = New York Botanical Garden; F = Field Museum of Natural History, Chicago, IL; CFH = Clark Fungal Herbarium, Worchester, MA.

portion of the Hawaiian island chain has been above water for 29 million years, so with potential island hopping, there is a possibility of the oldest age being around 29 my, not 5 my.]

Material and methods

Eleven Big Island, two Maui, and four Kauai *R. laulaha* specimens, a single Thai specimen (*Marasmius clavipes* ≡ *Rhodocollybia clavipes*), and a single Costa Rican collection of *R. lignitilis* J.L. Mata & Halling were sequenced for the ITS locus using the fungal specific ITS primers ITS1F and ITS4. The following thermocycler PCR settings were used: 94°C (1 minute), 50°C (45 seconds), 50 to 72°C ramp (1 minute), 72°C (1 minute), repeat 30 times, 72°C, (7 minutes) – (Vilgalys and Hester, 1990). PCR products were run on an agarose gel and excised bands were cleaned using gelase. Cycle sequencing was conducted using Big Dye v. 3.1. A 3730 ABI capillary sequencer was used for sequencing. Sequences were aligned with twenty-one GenBank sequences representing ten *Rhodocollybia* species and a *Gymnopus dryophilus* (Bull.) Murrill outgroup sequence (Table 1). Alignment was carried out using *Clustal X 1.83* (Thompson et al., 1994) software with further manual alignment using *MacClade v. 3.7* (Maddison & Maddison 1997). Phylogenetic reconstructions were performed using *PAUP 4.0b10* (Swofford 2000). A heuristic parsimony search and bootstrapping were conducted using a random stepwise addition with 1000 replicates. Of 1011 total characters, 561 ambiguously aligned characters were excluded from the analysis resulting in a total of 105 parsimony informative characters.

Additionally, a separate data set comprising six *R. laulaha* specimens (one Big Island, three Maui, and two Kauai), two *R. lignitilis* specimens from Panama and Costa Rica, a *R. unakensis* (Murrill) Halling specimen from Texas, and five GenBank sequences

Table 2. *Rhodocollybia* species included in the analysis of LSU sequence data.

Species	Herbarium*	Collection ID	Geographic Origin	GenBank Accession
R. badiialba	SFSU	DLL 9199	USA	AY639439
R. butyracea var. *asema*		GLM 46024	Germany	AY207163
R. butyracea var. *asema*	NY	REH 6705	USA	AY639440
R. laulaha	SFSU	DED 5873	Big Island, HI	AY639441
R. laulaha	F	MRK 120	Maui, HI	GU369960
R. laulaha	F	MRK 121	Maui, HI	GU369961
R. laulaha	F	MRK 123	Maui, HI	GU369962
R. laulaha	F	MRK 160	Kauai, HI	GU369963
R. laulaha	F	MRK 163	Kauai, HI	GU369964
R. lignitilis	NY	REH 7907	Panama	GU369965
R. lignitilis	TENN	56628	Costa Rica	GU369966
R. maculata	DU	RV94	USA	AF042597
R. maculata	CFH	AFTOL ID 540	USA	AY639880
R. unakensis	TENN	58545	Beaumont, TX	GU369967

* TENN = University of Tennessee; SFSU = Harry D. Thiers Herbarium, San Francisco State University; NY = New York Botanical Garden; F = Field Museum of Natural History, Chicago, IL; CFH = Clark Fungal Herbarium, Worchester, MA; DU = Duke University Fungal Herbarium.

representing three additional *Rhodocollybia* species was created for the 28S LSU locus using the 28S fungal specific primers LROR and LR6 (Table 2). PCR, sequencing, alignment, and analysis procedures were the same as for the ITS. The LSU internal primer LR3 was used in addition to the LROR and LR6 primers for sequencing. Of 835 total characters, 34 characters were parsimony informative. No positions in the alignment were ambiguous. Both data sets were also subjected to analysis using Bayesian methods (Ronquist & Huelsenbeck 2003) to obtain support statistics. Ten thousand trees resulted from 1,000,000 generations. Burn in was reached at 8500 trees.

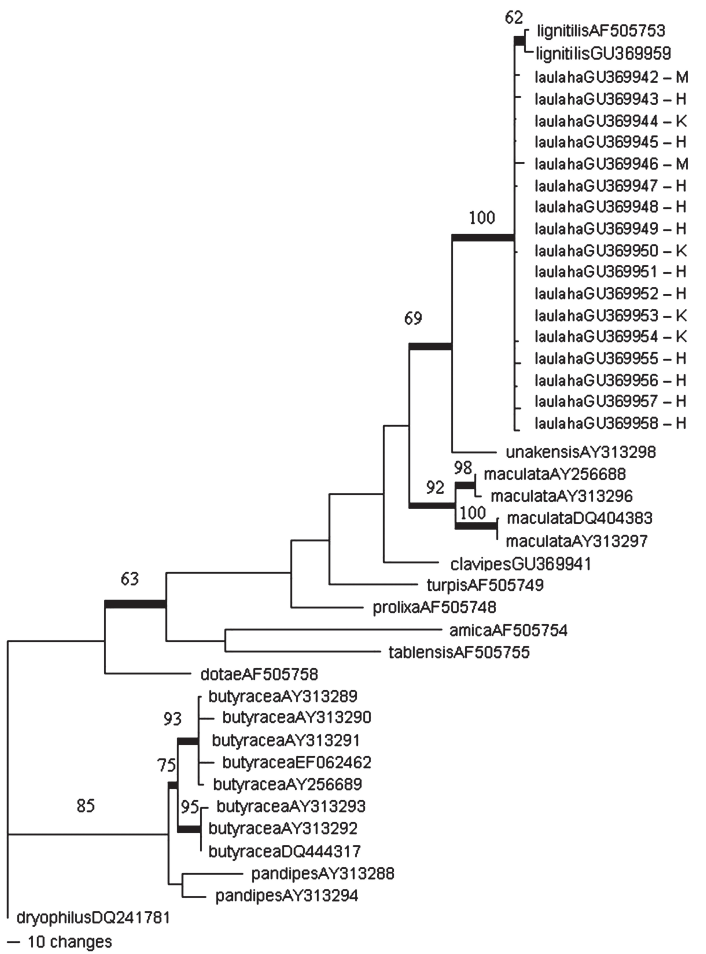

Figure 1. One of 52 equally most parsimonious trees of length 386 based on nuclear ribosomal ITS sequence data. Bootstrap support values greater than 60 appear above branches. Branches with Bayesian posterior probability values greater than 95% indicated as thickened branches. Island is indicated for seventeen *R. laulaha* collections (H=Big Island; M=Maui, K=Kauai).

Results

Both the ITS and LSU phylogenies (Figs. 1–2) indicate that *R. lignitilis* from Panama and Costa Rica is nested within *R. laulaha* of Hawaii with bootstrap support values of 100 and 99 respectively and greater than 95% Bayesian posterior probability support in each analysis. Their closest relative included in this analysis is *R. unakensis* from Texas; however, the *R. laulaha* clade is significantly diverged from other *Rhodocollybia* species. The *R. laulaha* clade is within the Maculata subclade (as distinguished from the Butyracea subclade in Mata et al. 2004). Variability within the ITS region is not sufficient to discern patterns within the *R. laulaha* clade across the Hawaiian Islands or even between Hawaiian individuals and the two collections from the neotropics. *Marasmius clavipes* of Thailand nests clearly within the genus *Rhodocollybia* and is formally transferred herein to *Rhodocollybia*.

Rhodocollybia clavipes (Corner) Desjardin & Keirle, **comb. nov**.

MycoBank MB516790

Basionym: *Marasmius clavipes* Corner, Beih. Nova Hedwigia 111: 42. 1996.

Type: Borneo, Mt. Kinabalu, Mesilau, 1700 m elev., RSNB 8180A (E!).

Additional material examined: Thailand, Chiang Mai Province, Doi Inthanon National Park, Hwy 1009 at junction with road to Mae Chem, 28 June 2007, *D.E. Desjardin 8151* (BBH, SFSU).

Discussion

It is perhaps not surprising that *R. lignitilis* appears to be conspecific with *R. laulaha* based on these molecular analyses. Despite the significant oceanic interruption in the species range, there are striking morphological similarities between the two taxa. Detailed examination of the protologues for *R. lignitilis* and *R. laulaha* indicates that the macromorphological and micromorphological features of the two are consistent and overlapping (cf. Desjardin et al., 1999 and Mata et al., 2004). As there are no fixed substitutions in the ITS of *R. lignitilis* that would permit reliable genetic differentiation between the two, it seems safe to declare them conspecific with the name *R. laulaha* having priority. Unfortunately, the neotropical population of *R. laulaha* is currently known from only two specimens: TENN 56628 from Costa Rica and R.E.H. no. 7907 from Panama. It is intriguing that a mushroom so common and so prolific in Hawaii has been collected on only two occasions in the neotropics, despite the fact that the specific collecting localities in Costa Rica and Panama from which it is known have been intensively sampled by mushroom biologists. Nonetheless, few mycologists focused on collecting *Rhodocollybia* in these areas and many of the *Rhodocollybia* described from Costa Rica and Panama are known from only a few specimens.

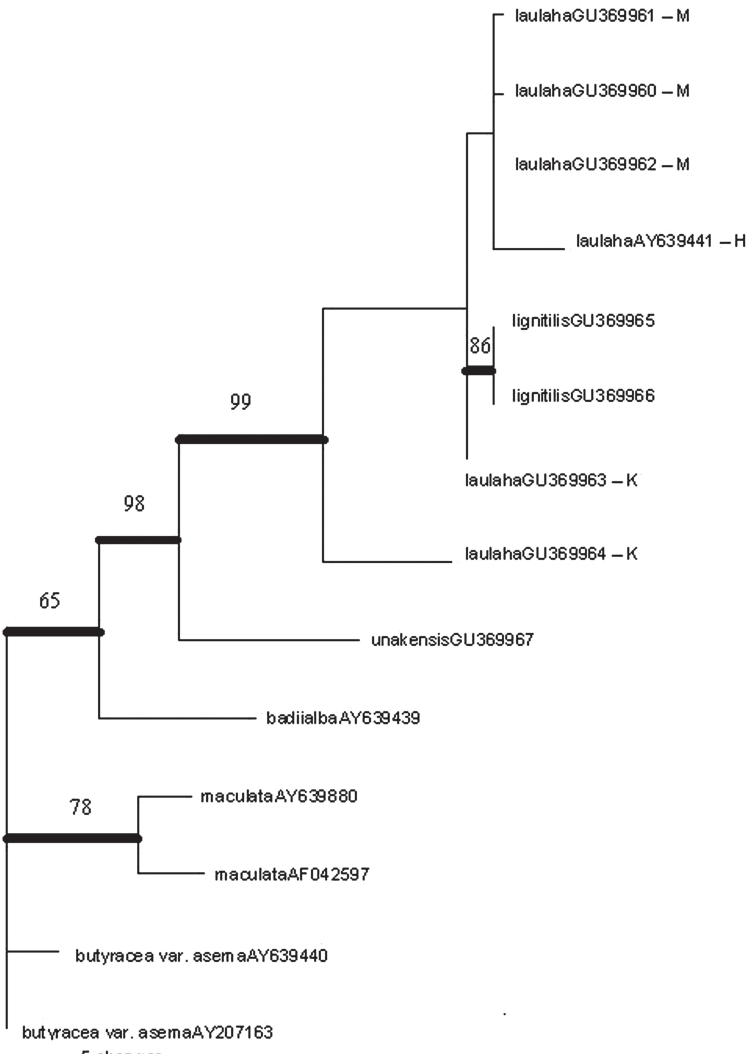

Figure 2. Single most parsimonious tree of length 71 based on nuclear ribosomal LSU sequence data. Bootstrap support values greater than 60 appear above branches. Branches with Bayesian posterior probability values greater than 95% indicated as thickened branches. Island is indicated for six *R. laulaha* collections (H=Big Island; M=Maui, K=Kauai).

Knowledge of this expanded range of *R. laulaha* into the neotropics allows for speculation about the biogeographic history of Hawaiian *Rhodocollybia*. The closest potential relative of *R. laulaha* (its sister taxon as recovered in the current analyses, which admittedly represents limited sampling) is *R. unakensis*. The specimen of *R. unakensis* used in this analysis was found near Beaumont, Texas (latitude 30.11°N), well within the North American subtropics. One cannot help but notice the connection between Hawaiian *Rhodocollybia* and *Rhodocollybia* in the Americas. The type collection from Unaka Springs, Tennessee, and the specimen we used in the analyses from Beaumont, Texas, are within the American subtropics. This would seem to be in line with many other Hawaiian taxa that also trace their ancestry to the New World (e.g. Baldwin & Robichaux 1995).

There are at least two straightforward scenarios that would explain the species distributions observed here. If *R. laulaha* originated in Hawaii, perhaps evolving from a New World ancestor that migrated west, the Costa Rican and Panamanian populations would represent relatively recent reverse migrations back to the Americas. If such a scenario were true, it might explain the relative lack of abundance of *R. laulaha* in the neotropics. Perhaps the oak forests of Central America provide a less than ideal habitat for this specialized Hawaiian endemic. Conversely, if *R. laulaha* originated in the New World and has only recently established in Hawaii, its rapid spread and colonization of Hawaiian endemic rain forests might reflect a case of 'ecological release' whereby constraints found in its native land are removed and it is able to expand its range and numbers with ease. Unfortunately, testing these conflicting hypotheses is not possible unless a considerable number of *R. laulaha* individuals can be collected from the neotropics. The latter scenario might appear more likely than the former in that it entails a single long distance migration event. However, without any way to assess the difficulty with which a mushroom species accomplishes such migration, it is impossible to argue that one migration event is any more likely than two events. Perhaps if appropriate genetic markers could be developed, a comparison of neutral and non-neutral markers or of synonymous and non-synonymous substitutions in a protein-coding marker might provide evidence in the Hawaiian *R. laulaha* populations of active positive selection or relaxed selection consistent with ecological release.

Clearly this investigation requires additional neotropical specimens. Ideally, with sufficient individuals representing the neotropics, multiple genetic markers might be able to determine current patterns of gene flow between Hawaii and the Americas (if realized) and the geography of origin – is *R. laulaha* Hawaiian or New World?

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